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**Process for Producing a Polyvinyl Alcohol Gel and a
Mechanically Highly Stable Gel Produced Thereby**

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TC 1700 MAIL ROOM

Application Cross Reference:

- 5 This application is the U.S. national phase of PCT application
PCT/DE99/00975 filed 30 March 1999, now WO 99/67320 with a claim to the
priority of German application 198 27 552.8 that was filed 20 June 1998.

Background of the Invention:

- 10 The present invention relates to a process for producing a polyvinyl alcohol
gel. The invention also refers to a mechanically highly stable gel produced
according to the process.

It is well known that solutions containing polyvinyl alcohol (PVA) become
more viscous when left to stand. It is also well known that PVA solutions can be
15 converted into a gel if the solution is frozen and subsequently thawed (FR 2 107
711 A). However, gels formed in this manner have a relatively low strength.

It is also known through EP 0 107 055 B1 that the strength of PVA gels
produced by freezing is increased by carrying out the freezing and thawing
process at least once, but preferably by repeating this process two to five times.

- 20 To this end, a PVA solution with a degree of saponification of ≥ 95 mol %, but
preferably of 98 mol %, is used. The upper temperature limit for freezing the
solution is preferably -3 degrees Celsius. The cooling rate can be between 0.1 to
50 degrees Celsius per minute. The thawing rate is preferably between 0.1 to 50
degrees Celsius per minute. The PVA that is used must have a degree of
25 polymerization of at least 700. The concentration of PVA in the solution should
be over 6 wt.% and is preferably between 6 and 25 wt.%. The PVA gel produced
by repeated freezing and thawing has a good mechanical strength and a high water
content, which is maintained even under mechanical stress. The gel produced in
this manner is highly elastic, non-toxic, and can be used in many applications,
30 particularly in medicine.

Various substances and materials can be added to the gel to increase the strength, including, but not limited to, glycol, glycerine, saccharose, glucose, agar, gelatine, methyl cellulose, etc. The admixture of agents such as heparin permits medical applications wherein the active agent is continuously and evenly released
5 from the gel over a period of time. In addition, microorganisms and enzymes can be mixed with the gel in order to create a biologically active system.

It is known from U.S. Patent No. 4,663,358 that adding organic solvents to the aqueous polyvinyl solution lowers the freezing point of the solution. This prevents the water from freezing at gelling temperatures below between -10 and
10 -20 degrees Celsius, thus forming a more homogeneous and transparent gel. The low gelling temperature is used for creating compact grained gels with sufficient mechanical strength.

Producing PVA gels using the freezing method is complicated and time consuming.

15 DE 43 27 923 C2 discloses a process by which PVA gels can be produced without using the freezing process. By using a PVA solution with a degree of hydrolysis of $\geq .99$ mol % and adding a dissolved additive that has non-aqueous OH or NH₂ groups, PVA gels can be produced at temperatures above zero (0) degrees Celsius. However, the gelling process has the drawback of taking up to
20 several hours to complete and it may even require an additional storage period of many hours in order to ensure that it is cured sufficiently to provide full strength to the gel substance. These drawbacks render the process disclosed in DE 43 27 923 C2 disadvantageous for producing large amounts of the gel substance.

The present invention is directed to overcoming one or more of the
25 problems set forth above.

Summary of the Invention:

An aspect of the present invention is to be able to produce the PVA gel substances simply and quickly. Another aspect of the present invention is to

improve the quality of the gel substance produces in this process as much as possible.

To achieve this aspect, the process for producing a polyvinyl alcohol gel includes the following steps:

- 5 a) Use of an aqueous polyvinyl alcohol solution with a degree of hydrolysis of ≥ 98 mol %;
- b) The addition of an additive that is dissolved in the aqueous polyvinyl alcohol solution and forms a separate, finely distributed and aqueous phase after concentration of the solution;
- 10 c) Dehydration of the aqueous solution up to a maximum residual water content of 50 wt.% in order to cause the phases to separate and hence the polyvinyl alcohol to gel; and
- d) Rehydration of the polyvinyl alcohol in an aqueous medium.

The above aspects are merely illustrative and should not be construed as 15 all-inclusive.

Brief Description of the Drawing:

Reference is now made more particularly to the drawing, which illustrates the best presently known mode of carrying out the invention and wherein similar 20 reference characters indicate the same parts throughout the view.

Fig. 1 is a graph showing the weight of the gel substances prior to and after the dehydration process for an example provided herein.

Detailed Description:

25 An aspect of the present invention is to be able to produce the PVA gel substances simply and quickly. Another aspect of the present invention is to improve the quality of the gel substance produces in this process as much as possible.

To achieve this aspect, the process for producing a polyvinyl alcohol gel 30 includes the following steps:

- 5
- a) utilizing an aqueous polyvinyl alcohol solution with a degree of hydrolysis of ≥ 98 mol%;
 - b) dissolving an additive in the aqueous polyvinyl alcohol solution;
 - c) dehydrating the aqueous solution to a maximum residual water content up to 50 wt.% in order to cause the phases to separate and the polyvinyl alcohol to gel with the additive forming a separate, distributed and aqueous phase; and
 - d) rehydrating the polyvinyl alcohol in an aqueous medium.

Surprisingly, the process according to the invention permits the gelling of
10 the polyvinyl alcohol within several minutes at room temperature or even higher temperatures. The addition of the water-soluble additive and the concentration by evaporation of the water results in a finely distributed phase separation, whereby gelling happens within a very short period of time during the PVA phase. The water-soluble additive advantageously creates a hydrous phase, so that within a
15 very short period of time, a respective proportion of water is removed through the phase separation of the PVA phase, thus achieving the gelling of the polyvinyl alcohol. It is advantageous if the water-soluble additive has an affinity to water at least comparable to that of the PVA or greater.

The PVA phase, which is under-supplied with water during the gelling
20 process, subsequently absorbs water during rehydration, while the elasticity and mechanical strength of the PVA gel is improved without reversing the gelling. It has been shown that a certain amount of electrolytes in the aqueous rehydration medium results in a higher stability of the PVA gel. Thus, the rehydration may advantageously be performed in tap water or, better yet, in a saline solution.

25 The process according to the invention has the advantage that it permits the production of PVA gel within a very short time without using complicated processes and, in particular, without a freezing process and without repeated dehydration processes, thus allowing for an extremely economical production of PVA gel. The gel substances according to the invention are also characterized by

a high elasticity and stability, in particular tensile strength, and in this regard are significantly superior to the PVA gel substances produced in the traditional way.

The stability and elasticity of the PVA gel substances are further enhanced by using a resaponified aqueous PVA solution in the production process.

5 Polyethylene glycol is a preferred water-soluble additive that is added at a concentration of 4 to 30 wt.%, preferably at 4 to 20 wt.%, and more preferably at 6 to 16 wt.%. Cellulose esters, cellulose ethers, starch esters, starch ethers, polyalkylene glycol ethers, polyalkylene glycols, long-chain alkanoles ($C_nH_{2n+1}OH$ where $n \geq 8$), sugar esters and sugar ethers are other examples of
10 possible additives.

A particularly advantageous area of application of the PVA gel substances is their production as biologically, physically or chemically active substances, i.e. incorporating biologically, physically or chemically active material in the PVA gel. For example, the PVA gel is superbly suited for the production of chemical
15 or biological catalysts.

The dehydration of the aqueous solution for the purpose of phase separation and the associated gelling is performed until a maximum residual water content of 50 wt.% is reached. A lower limit for the residual water content is 10 wt.% to allow for the rehydration of the PVA gel substance to avoid a reduction in
20 the elasticity of the gel substance. This lower limit of the residual water content is also preferable because incorporated biological materials can be damaged if the residual water content is lower than 10 wt.%. An advantageous range for the residual water content is between 10 and 30 wt.%.

Dehydration can conveniently be performed in a short period of time by
25 evaporating water at ambient air temperature, provided the aqueous solution is divided into small portions, especially portions wherein there is only a small proportion of starch in the solution. In particular, it is advantageous to drip the solution onto a hard surface in a way such that the diameter of the drop is at least twice the height of that drop. This can similarly be achieved by pouring the
30 solution into a form and/or coating a base material. A thin or even film-like shape

permits evaporation to the required residual water content within a matter of several minutes. An acceleration of the dehydration process (and, therefore, the gelling process) can be achieved by carrying out dehydration in a drying oven at a higher temperature.

- 5 The saline solution used advantageously for rehydration preferably contains polyvalent anions.

The process according to the invention is particularly advantageous for immobilizing biologically active material in that it is extraordinarily gentle on the biological material. Thus, the biological material has a significantly higher initial
10 activity in comparison with other immobilization processes.

This can be enhanced if the aqueous medium serves as a means to rehydrate the PVA, as well as a culture solution for the biologically active material.

The density of the PVA gel produced according to the invention can be
15 modified by using suitable additives. For example, the specific gravity can be increased by adding titanium oxide and lowered by adding hollow glass microballs.

As already mentioned, gelling according to the invention is possible at room temperature but can also be performed at lower or higher temperatures. The
20 biologically active material that is incorporated into the PVA gel can be, among other things, enzymes, microorganisms, spores and cells.

The process according to the invention can be implemented in various embodiments. For instance, it is possible to carry out the dehydration of a drop for phase creation during the falling process in a drop tower, such that when the
25 drop impacts the surface, the gelling has already occurred after the phase separation. This production process is particularly suited for producing PVA gel substances as chromatography material whose diameter is 10 to 100 µm for laboratory purposes, but may otherwise be between 100 and 800 µm. It is also possible to dehydrate an initial liquid that is set to a higher viscosity during
30 extrusion of a strand and to carry out the gelling simultaneously.

Compared to previously known gel substances, the gel substance produced by the process according to the invention has superior mechanical stability, particularly with regard to abrasion resistance and tensile strength.

- These superior mechanical characteristics permit production of the gel
- 5 substance according to the invention in a lenticular form that is advantageous to the kinetics of the reaction, where previously known gel substances had insufficient mechanical stability, most notably agitation stability. Furthermore, the gel substance according to the invention is stable and abrasion resistant for many months, even when subject to high-speed agitation. The lenticular form
- 10 with a large diameter and low height results in the arrangement of the chemically, physically and biologically active material near the surface, allowing for a constellation that is advantageous for the kinetics of the reaction.

The present invention also permits the addition of a magnetic additive to the polyvinyl alcohol substance by a very simple method in order to collect the gel

15 substance out of a liquid by means of magnets.

It has been shown that the pore structure of the polyvinyl alcohol gel substance can be controlled by the molecular weight of the additive that effects the phase separation. By varying the molecular weight of the added polyethylene glycol, whose molecular weight is preferably in the range of 800 and 1350, the

20 pore size of the polyvinyl alcohol gel substance can be adjusted to between 1 and 15 μm .

In the production of the aqueous solution of polyvinyl alcohol and the additive, it has been shown that the use of distilled water requires a higher degree of dehydration in order to achieve the same mechanical characteristics. The

25 results are immediately improved if normal tap water with a certain degree of hardness is used. Therefore, it can be assumed that the presence of salt in the water is advantageous for the process according to the invention.

The invention is explained in more detail below by means of examples of embodiments.

Example 1:

16.8 g. of water are added to 2 g. of PVA and 1.2 g. of polyethylene glycol (PEG 1000). The solution is heated to 90 degrees Celsius until all the components have dissolved completely, so that a viscous, colorless solution is obtained. After 5 cooling to 30 degrees Celsius, the polymer solution is dripped onto a polypropylene plate by means of a syringe and application of pressure. The dripping is performed by touching the cannula onto the PP plate at a rate of approximately 1-2/second; the drop has a diameter of approximately 3 mm and a height of approximately 1 mm. After application of the drops, a white, wax-like 10 film forms on the surface of the drops. After 89 wt.% of the water has evaporated at room temperature, the gel substances are rehydrated in water or a saline medium. The gel substances obtained have a diameter of 3-4 mm and a height of approximately 200-400 μm .

15 Example 2:

After the polymer suspension (composition: 2 g. PVA, 1.2 g. PEG 1000 and 15.8 g. water) has cooled, 1 ml of a nitrogen-fixing mixed culture (Nitrosomonas europaea and Nitrobacter winogradsky) is added to 20 g. of a polymer solution and dispersed, resulting in a dry biomass load of 0.06 wt.%. The 20 production of the gel substances is performed according to Example 1. The gel substances obtained are rehydrated in a standard mineral salt medium for nitrogen-fixers. Compared to the same amount of free nitrogen fixers, after immobilization the immobilizates produced in this way have an initial activity of approximately 70% for Nitrosomonas spp. and 100% for Nitrobacter spp.

25 After occlusion of the nitrogen fixers in the PVA kyrogels at -20 degrees Celsius, the initial activity of Nitrosomonas spp. is approximately 1%, at -10 degrees Celsius approximately 25% with decreasing stability of the PVA hydrogels.

The incubation of the immobilizates is performed in the same medium at 30 30 degrees Celsius. After 19 days a maximum ammonium decomposition rate of

between 7 and 8 $\mu\text{mol NH}_4^+ / (\text{g}_{\text{cat}} \times \text{min})$ is achieved if 10 mg of gel substance is incubated in 30 ml of standard mineral salt medium.

Example 3:

5 1.6 g. of polyethylene glycol (PEG 1000) is dissolved in 12.8 g. H₂O, followed by 1.6 g. of PVA. The process is continued according to Example 1. After the polymer solution has cooled to 30 degrees Celsius, 4 ml of a culture of the strictly anaerobic bacteria Clostridium butyricum NRRL B-1024, which converts glycerine into 1.3-propane diol (PD), grown overnight in an oxygen-free 10 atmosphere is dispersed in the solution (cell load of the polymer solution: 6×10^7 per ml). The gel substances are produced according to Example 1. After 70 wt.% of the water has evaporated at room temperature, the immobilizes are rehydrated in a mineral salt medium (20 times more than required). The incubation of the cell-loaded gel substance is performed in the same medium (40 times more than 15 required) at 30 degrees Celsius. The medium is changed several times during the growing phase in order to provide the immobilized biomass with an adequate nutrient supply.

If 0.25 g. of the immobilized bio-catalyst thus obtained are placed in 40 ml of mineral salt medium together with 24.4 g. L-1 glycerine, within 3.25 h the 20 concentration of 1.3-PD increases by 2.8 g. L-1. This corresponds to a catalyst activity of 0.14 g. 1.3-PD per g. cat per hour. After deducting the activity of fully developed cells, the result is a catalyst activity of 0.08 g. 1.3-PD ($\text{g}_{\text{cat}} \times \text{h}$).

Example 4:

25 15.8 g. of water is added to 2 g. PVAL and 1.2 g. polyethylene glycol (PEG 1000) and the process is continued according to Example 1.

After cooling the polymer suspension to approximately 30-37 degrees Celsius, 1 ml of a defined spore suspension of the fungus Aspergillus terreus is added to a polymer solution of 20 g. and dispersed. The spore suspension is

selected so that 5 d of fouling in the growth medium results in a dry biomass load of 0.05 wt.%.

After 70 wt.% of the water has evaporated at room temperature, the immobilizates are rehydrated in a mineral salt medium for *Aspergillus terreus* (20 times more than required).

The incubation of the immobilizates is performed in the growing medium. The growing medium is replaced with production medium for producing the itaconic acid.

Compared to the same amount of free fungus cells, the immobilizates thus produced have an initial activity of approximately 60% directly after immobilization. If 0.2 g. of gel substance is incubated in 100 ml of production medium together with 60 g./l. glucose, a productivity of 35 mg of itaconic acid ($g_{cat} \times h$) is achieved after 7 d.

15 Example 5:

Larger amounts of gel substances are obtained by dripping the polymer solution (composition according to Example 1) through a system of multiple nozzles onto a conveyer belt. According to the principle of a belt dryer, the PVA drops are dehydrated in a tunnel drier to a defined residual moisture content and subsequently collected in a collection container by means of a scraper, where they are rehydrated and washed.

Example 6:

In the production process according to Example 1, the polymer solution is not dripped, but poured into prefabricated semi-open forms of optional length with an interior diameter of 1-10 mm.

After rehydration in water, the strands can be stretched to 3-4 times their length without breaking. This stretching is irreversible. A strand produced in this way can be loaded with a weight of 500 g. without breaking.

Example 7:

After a storage period of 14 days, the strands produced according to Example 6 are mechanically characterized in tap water. At that time, the strands are approximately 8 mm wide and approximately 1 mm high. The degree of rehydration takes into account the strand's loss of weight after rehydration and 14 d storage in water in relation to the total mass of the polymer solution prior to the dehydration process. The strands have an elastic behavior up to an elongation at tear of 40%.

Mechanical characterization of the produced strands with various degrees of dehydration for the composition 10 wt.% PVA and 6 wt.% PEG 1000:

| | Residual water content after dehydration [%] | Degree of rehydration [%] | Elongation at tear [%] | E-modulus [N/mm ²] |
|----|--|---------------------------------|---------------------------|-----------------------------------|
| 15 | 27 | 76 | 455 | 0.11 |
| | 20 | 74 | 420 | 0.11 |
| | 15 | 68 | 410 | 0.18 |
| | 13 | 65 | 390 | 0.24 |
| | 10 | 63 | 380 | 0.27 |
| | 1 | 57 | 360 | 0.34 |

Mechanical characterization of the strands at a degree of dehydration of 80 wt.% for the composition 10 wt.% PVA and 8 wt.% PEG for various types of PEG:

| | Type of PEG | Degree of rehydration [%] | Elongation at tear [%] | E-modulus [N/mm ²] |
|----|-------------|---------------------------------|---------------------------|-----------------------------------|
| 25 | 400 | 57 | 410 | 0.27 |
| | 600 | 66 | 290 | 0.22 |
| | 800 | 82 | 360 | 0.19 |
| | 1000 | 84 | 420 | 0.11 |
| | 1350 | 92 | 370 | 0.12 |

Mechanical characteristics of the PVA hydrogel strands for various concentrations of PVA with the addition of 6 wt.% PEG 1000 at a degree of dehydration (amount of water evaporated during the dehydration process) of 80 wt.%:

| | PVAL [%] | Elongation at tear [%] | E-modulus [N/mm ²] |
|---|-------------|---------------------------|-----------------------------------|
| 5 | 8 | 350 | 0.09 |
| | 10 | 420 | 0.11 |
| | 12 | 420 | 0.17 |
| | 14 | 460 | 0.19 |
| | 16 | 440 | 0.25 |

Mechanical characteristics of the strands at a degree of dehydration of 80 wt.% for the composition 10 wt.% PVA and 6 wt.% PEG 1000 for various rehydration media:

| | Rehydration medium | Elongation at tear [%] | E-modulus [N/mm ²] |
|----|--|---------------------------|-----------------------------------|
| 15 | Tap water | 420 | 0.11 |
| | K ₂ HPO ₄ (100 mmol/l) | 410 | 0.17 |
| | K ₂ SO ₄ (120 mmol/l) | 530 | 0.15 |
| | CaCl ₂ (120 mmol/l) | 360 | 0.10 |
| | KCl (175 mmol/l) | 370 | 0.15 |

20

Example 8:

Gel substances are produced according to Example 1 and rehydrated in deionized water (5 µS H₂O). The degree of rehydration for various degrees of dehydration of the gel substances is defined directly after the rehydration process.

With a degree of rehydration of 100 wt.%, the weight of the gel substances prior to the dehydration process and after the dehydration process is the same, as shown in Fig 1.

Other objects, features and advantages will be apparent to those skilled in the art. While preferred embodiments of the present invention have been illustrated and described, this has been by way of illustration and the invention should not be limited except as required by the scope of the appended claims.